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Development and validation of High Performance Thin Layer Chromatography for estimation of Cefpodoxime Proxetil and Dicloxacillin sodium in combined tablet dosage form

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Abstract

High performance thin layer chromatography for estimation of Cefpodoxime Proxetil and Dicloxacillin Sodium in their combine dosage form was developed and validated. The method was performed on Camag HPTLC Instrument using Precoated silica gel 60 F254 Aluminum plates (20×10cm) as stationary phase and Benzene: Chloroform : Methanol (5:3:2) as mobile phase at ambient temperature. Detection was carried out at 220 nm. Linearity was observed at concentration range 300-1800ng per band for Cefpodoxime Proxetil and 1500-9000 ng per band for Dicloxacillin Sodium. Correlation coefficient for Cefpodoxime Proxetil and Dicloxacillin Sodium was found 0.996 and 0.999 respectively. Retention factor was found to be 0.24 for Cefpodoxime Proxetil and 0.62 for Dicloxacillin Sodium. The method can successfully applicable to routine analysis.

Keywords: Cefpodoxime Proxetil, Dicloxacillin Sodium, HPTLC

Contents

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1. Introduction

[1, 2]Cefpodoxime Proxetil is Third Generation Cephalosporin Antibiotic and it is chemically (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid . Dicloxacillin Sodium is Anti-Bacterial -One of the penicillins which is resistant to penicillinase and chemically it is (2S,5R,6R)-6-[3-(2,6-dichlorophenyl)-5-methyl-1,2-oxazole-4-amido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.[3] Structure of Cefpodoxime Proxetil is illustrated in fig.1. Structure of Dicloxacillin Sodium is illustrated in fig.2.

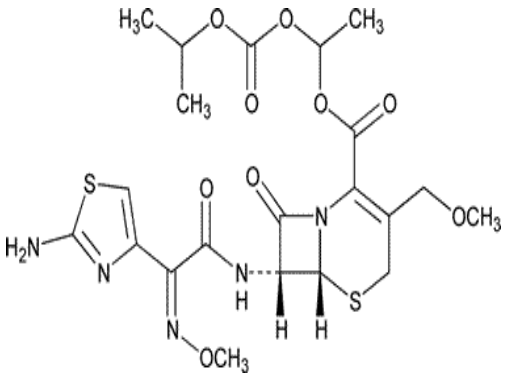

Fig 1. Structure of Cefpodoxime Proxetil

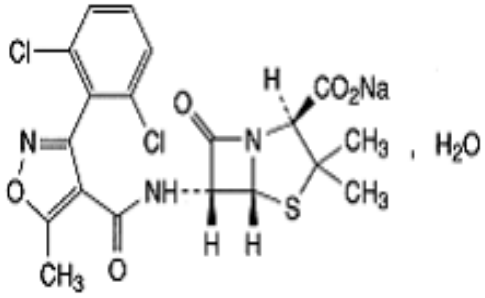

Fig 2. Structure of Dicloxacillin Sodium

So far, to our present knowledge, no single HPTLC method for estimation of Cefpodoxime Proxetil and Dicloxacillin Sodium has been reported.[4-10] So an attempt was made to develop single, accurate, rapid, and precise HPTLC method for the determination of Cefpodoxime Proxetil and Dicloxacillin Sodium in tablet and in active pharmaceutical ingredients.

2. Material and methods

Instruments:

CAMAG HPTLC instrument was used in this method. CAMAG HPTLC is equipped with CAMAG TLC scanner-3, Linomate V automatic sample applicator controlled by WIN CATS software (1.4.3 version)

Reagent and chemicals:

Cefpodoxime Proxetil and Dicloxacillin Sodium was a gift sample from Baroque pharmaceutical Limited, Khambhat. All chemicals and reagent used were analytical grade and purchased from Ranbaxy fine chemicals Limited. Combined tablet formulations (Zedocef-DXL-200) were procured from Indian market.

Preparation of standard stock solution:

Accurately weigh 50 mg of Cefpodoxime Proxetil and Dicloxacillin Sodium in 50ml volumetric flask and make up the volume with methanol which give final strength about 1000µg/ml.

Selection of wavelength

Appropriate volume of Cefpodoxime Proxetil and Dicloxacillin Sodium about 1ml and 5ml were respectively taken in 50ml volumetric flask and volume was made up to mark with methanol. The resulting solution was scanned in UV range (200 nm- 400nm). In the spectrum of Cefpodoxime Proxetil and Dicloxacillin Sodium 220 nm is selected because at this wavelength both drugs absorb UV radiation

Optimized chromatographic conditions:

Stationary phase: Precoated silica gel 60 F254 Aluminium plates (20×10cm)
Thickness of plates: 0.2mm (E.MERCK)
Mobile phase: Benzene: Chloroform: Methanol (5:3:2)
Chamber saturation time: 20 minutes
Development distance: 75mm
Development time: 15 minutes
Relative temperature: 25±2ºC
Relative Humidity: 44-49%
Detection wavelength: 220nm
Distance between two tracks: 10 mm

Preparation of calibration curve:

To construct calibration curve 3,6,9,12,15,18 µl solution of mixture of Cefpodoxime Proxetil and Dicloxacillin Sodium which is equivalent to 300, 600, 900, 1200 ,1500 and 1800ng/band of Cefpodoxime Proxetil and 1500,3000,4500,6000,7500 and 9000 ng/band of Dicloxacillin Sodium were applied to three plates.

The plate were developed and scanned at 190 and 360nm and peak areas were recorded for Cefpodoxime Proxetil and Dicloxacillin Sodium. Linear regression data for the calibration plots (n=6) are illustrated in fig no 3 and 4 respectively.

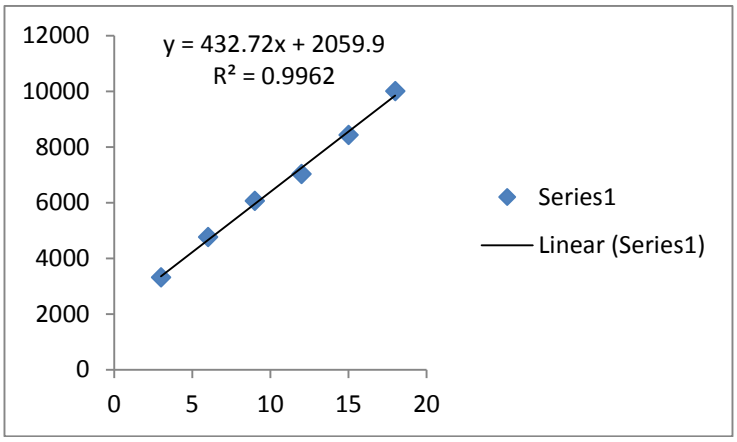

Figure 3. Calibration curve of Cefpodoxime Proxetil

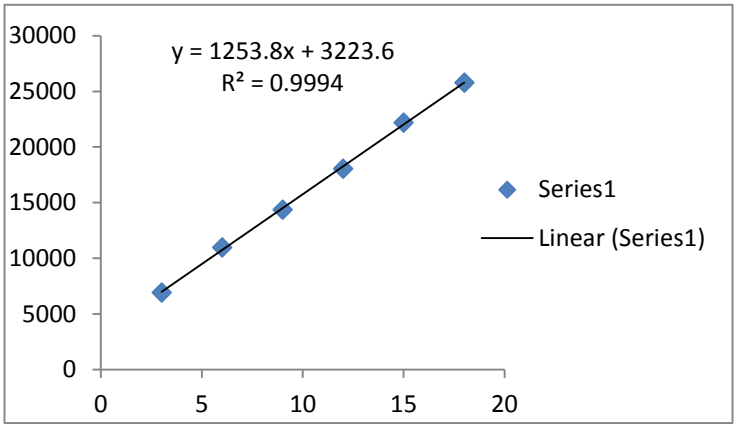

Figure 4. Calibration curve of Dicloxacilin Sodium

Analysis of commercial formulation:

Twenty tablets were weighed and the average weight was calculated a quantity of mixed content of 20 tablets equivalent to 100mg and 500 mg of Cefpodoxime Proxetil and Dicloxacillin Sodium was accurately weighed and transferred in to 100 ml volumetric flask. The solution is dilute up to 100 ml with methanol.

Than sample solution 10µl was applied to plate with standard solutions. The plate were developed and scanned under the optimized conditions as describe above. Peak areas were recorded and amount of Cefpodoxime Proxetil and Dicloxacillin Sodium in the formulation was determined by use of calibration curve.

Application of sample:

The TLC plates were prewashed with methanol and activated by keeping at 115 ºC for about 30 minutes. Aliquots were applied on the precoated silica gel G60 F254 TLC plates. Sample was spotted in the form of band of width 10mm with Hamilton micro liter syringe with slit dimensions about 6× 0.45mm. Distance between bands was 10mm.

3. Result and discussion

The method was validated by establishing linearity, accuracy, interday and intraday precision of measurement of sample application. The limit of detection and limit of quantification were also determined. [11,12]

Linearity calibration curve:

Calibration curve were found to be linear in the range of 300-1800 ng/band of Cefpodoxime Proxetil and 1500-9000 ng/band of Dicloxacillin Sodium. Five concentration points were assayed in triplicate. Both Cefpodoxime Proxetil and Dicloxacillin Sodium showed good linearity in tested range. The regression coefficient (R2) Value for Cefpodoxime Proxetil and Dicloxacillin Sodium were found to be 0.996 and 0.999 respectively. Individual chromatogram of Cefpodoxime Proxetil and Dicloxacillin Sodium is shown in figure no 5,6 respectively. Linearity of Dicloxacillin Sodium and Cefpodoxime Proxetil is shown in figure no 7.

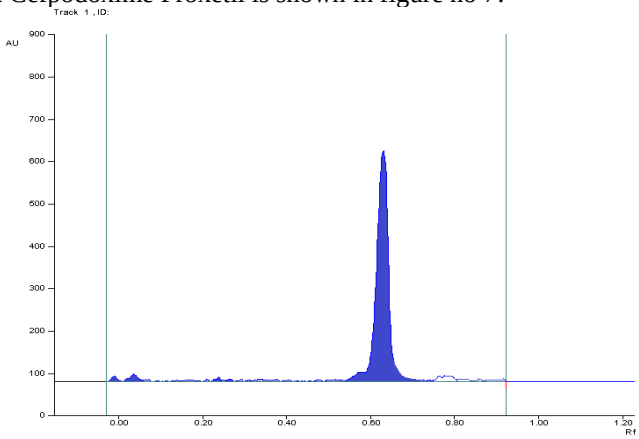

Figure 5 Chromatogram of Cefpodoxime Proxetil

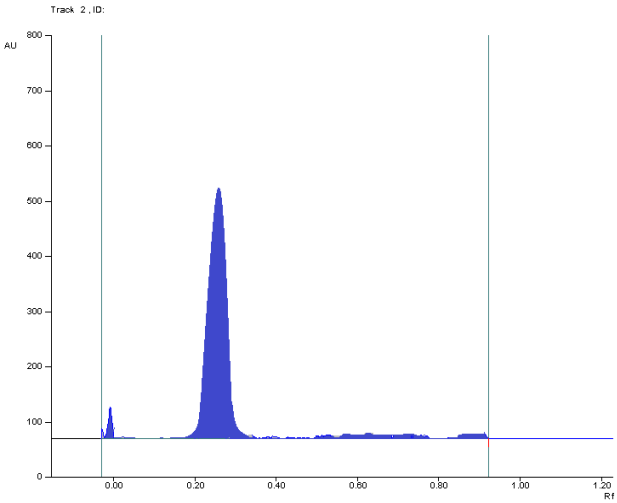

Figure 6. Chromatogram of Dicloxacillin sodium

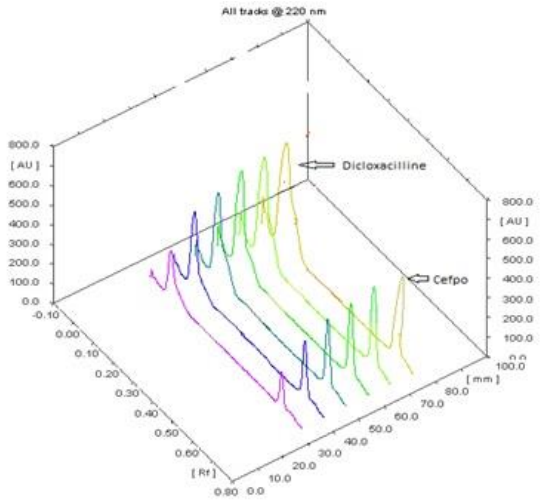

Figure 7. Linearity of Dicloxacillin Sodium and Cefpodoxime Proxetil

Accuracy:

Recovery study was carried out for accuracy parameter. The study was carried out at three level. To powder formulation the standard drug Cefpodoxime Proxetil and Dicloxacillin Sodium were added 80%, 100%,120% levels, dilution were made and analyzed by the method. The % recovery and %RSD were calculated and found to be within the limit as shown in table no 1.

Table no 1. Result for recovery study for Cefpodoxime Proxetil and Dicloxacillin sodium.

Level	Concentration (ng/spot)		Spike solution		Area of spike solution		% Assay	
	Cef	Dic	Cef	Dic	Cef	Dic	Cef	Dic
80%	900	4500	720	3600	5168.3	12321.7	99.80	100.81
80%	900	4500	720	3600	5174.3	12311.9	100.00	100.71
80%	900	4500	720	3600	5166.7	12300.1	99.75	100.58
100%	900	4500	900	4500	5981.2	14494.7	100.72	99.91
100%	900	4500	900	4500	5987.4	14592.3	100.88	100.78
100%	900	4500	900	4500	5988.6	14497.6	100.91	99.94
120%	900	4500	1080	5400	6772.1	16981.5	100.85	101.63
120%	900	4500	1080	5400	6742.3	16723.4	100.22	99.72
120%	900	4500	1080	5400	6741.9	16732.2	100.21	99.79

Precision:

Intraday precision was found by analysis of standard drug at six times on the same day. While interday assay precision was carried out on six different day. The RSD was found to be less than 2 for both interday precision and intraday precision. Result for the interday precision and intraday precision is shown in table no 2, 3 respectively.

Table no 2. Results for interday Precision

Concentration(ng/spot)		Area		% Assay	
Cefpodoxime Proxetil	Dicloxacillin Sodium	Cefpodoxime Proxetil	Dicloxacillin Sodium	Cefpodoxime Proxetil	Dicloxacillin Sodium
900	4500	5982.6	14499.7	100.75	99.96
900	4500	5987.9	14501.2	100.89	99.97
900	4500	5988.3	14512.3	100.90	100.07
900	4500	5990.2	14498.4	100.95	99.95
900	4500	5992.5	14495.8	101.01	99.92
900	4500	5989.4	14532.3	100.93	100.25
			Average	100.90	100.02
			SD	0.09	0.12
			%RSD	0.08	0.12

Table no 3. Results for Intraday precision

Concentration(ng/spot)		Area		% Assay	
Cefpodoxime Proxetil	Dicloxacillin Sodium	Cefpodoxime Proxetil	Dicloxacillin Sodium	Cefpodoxime Proxetil	Dicloxacillin Sodium
900	4500	6002.5	14530.2	101.26	100.23
900	4500	5987.3	14499.8	100.87	99.96
900	4500	5992.4	14504.7	101.00	100.00
900	4500	5997.1	14498.2	101.12	99.94
900	4500	5996.9	14502.6	101.12	99.98
900	4500	5994.3	14489.9	101.05	99.87
			Average	101.07	100.00
			SD	0.13	0.12
			%RSD	0.13	0.12

Table no 4. Summary of validation parameters

Sr. No	Validation parameter		Cefpodoxime Proxetil	Dicloxacillin Sodium
1	Linearity	Range Correlation coefficient	300-1800 ng/spot 0.996	1500-9000 ng/spot 0.999
2	Sensitivity	Limit of quantification	8.45ng/spot	18.23ng/spot
		Limit of detection	6.65ng/spot	20.14ng/spot
3	Precision (%RSD)	Method precision (repeatability) Intermediate Precision	0.08 0.13	0.12 0.12
4	Accuracy	% recovery	100.21	99.79
5	% Assay	% amount of drug found in tablet	99.96%	100.01%

4. Conclusion

A relatively simple HPTLC method was optimized and validated with system suitability for the simultaneous determination of the Cefpodoxime Proxetil and Dicloxacillin Sodium according to the ICH guidelines. The validation data indicate good precision, accuracy and reliability of the method. The developed method offers several advantages in terms of simplicity in mobile phase, retention time, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in simultaneous determination of Cefpodoxime Proxetil and Dicloxacillin Sodium in tablet dosage forms. Summary of validation parameters is shown in table no 4

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